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Localization of pheromonal sexual dimorphism in *Drosophila melanogaster* and its effect on sexual isolation

(pheromones/cuticular hydrocarbons/reproductive isolation/speciation)

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ABSTRACT ***Drosophila melanogaster* is sexually dimorphic for cuticular hydrocarbons, with males and females having strikingly different profiles of the long-chain compounds that act as contact pheromones. Gas-chromatographic analysis of sexual mosaics reveals that the sex specificity of hydrocarbons is located in the abdomen. This explains previous observations that *D. melanogaster* males display the strongest courtship toward mosaics with female abdomens. We also show that males of the sibling species *Drosophila simulans* preferentially court *D. melanogaster* mosaics with male abdomens. Because the primary male hydrocarbon in *D. melanogaster* is also the primary female hydrocarbon in *D. simulans*, this supports the idea that interspecific differences in cuticular hydrocarbons contribute to sexual isolation.**

The cuticle of *Drosophila*, like that of other insects, is covered with a layer of lipids and long-chain hydrocarbons. These serve several functions, including protection against desiccation and stimulation of male courtship (1). Recent work has concentrated on the pheromonal function of these compounds and the genetic bases of intraspecific polymorphisms and interspecific differences (2–6). In several species of *Drosophila*, large cuticular hydrocarbons act as female contact pheromones detected by the male's tapping or licking of the female during courtship (7, 8).

Little is known about the developmental biology of these hydrocarbons. They are thought to result from the synthesis of fatty acids from two-carbon moieties, followed by oxidative decarboxylation and reduction to unsaturated compounds (7, 9–11). Several genes have been mapped that cause intraspecific polymorphism and interspecific differences in *Drosophila* hydrocarbons (12–15), but the gene products have not been identified and their role in hydrocarbon synthesis is unknown.

Three studies of sexual behavior have led to the hypothesis that the abdomen plays an important role in production of *Drosophila* pheromones (16–18). Using a method pioneered by T. H. Morgan's group (19, 20), these investigators created gynandromorphs (sexual mosaics) of *Drosophila melanogaster*, which they exposed to nonmosaic males of the same species. (As described below, this species is sexually dimorphic for hydrocarbons.) All three studies showed that males will not court mosaics unless they have some female abdominal tissue, and the gender of other parts of the body is irrelevant. These authors speculated that the abdomen may therefore be the main site of pheromone synthesis or emission. This speculation is further supported by evidence that cuticular hydrocarbons are synthesized in the abdomen of mosquitoes and houseflies (10, 11, 21, 22). Up to now, however, there has been no direct evidence that the abdomen plays a role in synthesis of *Drosophila* hydrocarbons; as Nissani (17) notes, the attractiveness of the female abdomen to males could be due to factors other than pheromones (e.g., its morphology). Moreover, because the *Drosophila* mosaics were tested alive, one cannot rule out the possibility that males are attracted by the behavior and not the hydrocarbons of mosaics with female abdomens.

Here we demonstrate, through direct measurement of hydrocarbons, that the gender of the abdomen is indeed the sole determinant of whether or not a fly produces male or female pheromones. In addition, to test the idea that pheromonal differences among species contribute to sexual isolation, we conducted courtship experiments with gynandromorphs, presenting them not to *D. melanogaster* males but to males of the sibling species *Drosophila simulans*.

D. simulans is one of eight sibling species in the *D. melanogaster* subgroup that have been analyzed for pheromones and sexual isolation against their relatives. *D. melanogaster* is sexually dimorphic for hydrocarbons, with males having high levels of the 23-carbon compound *cis*-7-tricosene (7-T). *D. melanogaster* females have very little 7-T but high levels of two other hydrocarbons: the 27-carbon compound *cis,cis*-7,11-heptacosadiene (7,11-HD) and the 29-carbon compound *cis,cis*-7,11-nonacosadiene (7,11-ND) (5, 23, 24). *D. simulans*, on the other hand, is sexually monomorphic, with both sexes having 7-T as the predominant hydrocarbon (5, 14). Some African populations of both species have high levels of other hydrocarbons (5, 25).

Two of these hydrocarbons are known to induce male courtship within species: 7,11-HD in *D. melanogaster* and 7-T in *D. simulans* (7). The idea that interspecific differences in hydrocarbons play a role in sexual isolation was suggested by Cobb and Jallon (26) after comparing interspecific mating behavior with interspecific differences in pheromones among eight species of the *D. melanogaster* subgroup. These workers observed that males of species sexually monomorphic for hydrocarbons (both sexes having 7-T) court females only from other monomorphic species, while males of sexually dimorphic species (males with 7-T, females with 7,11-HD) court females of all eight species. Cobb and Jallon (26) suggested that this asymmetry was based on hydrocarbons, proposing that a male will court a heterospecific female only if she shares either his own predominant hydrocarbon or that of his conspecific females. This hypothesis was recently supported by experiments that transferred hydrocarbons among females of different species. These exchanges affected the females' attractiveness to males in precisely the way predicted by Cobb and Jallon (14).

Our production of *D. melanogaster* mosaics allows us to further explore the role of pheromones in sexual isolation by testing gynandromorphs against male *D. simulans*. If pheromonal differences cause sexual isolation between these species, we expect that *D. simulans* males will preferentially court those mosaics with low levels of 7,11-HD but high levels of 7-T. We

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Abbreviations: 7-T, 7-tricosene; 7,11-HD, *cis,cis*-7,11-heptacosadiene; 7,11-ND, *cis,cis*-7,11-nonacosadiene.

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therefore predict that mosaics will be courted most intensively if the body parts responsible for the sexual dimorphism are male. This prediction enables us to separate the effects of morphology from those of hydrocarbons. Moreover, the use of freshly killed instead of live mosaics in this experiment eliminates the potentially confounding factor of female behavior in eliciting male courtship.

METHODS AND MATERIALS

Fly Stocks. *D. simulans*: an isofemale line collected in Florida City, FL, in 1985 and used in our previous mating experiments (27–29).

(*ii*) *D. melanogaster* Horka: *Fs(3)* Horka, *mwh, e/T(1:3) OR 60/TM3, y*$^{+}$ *re p*^{p} *sep l(3)89Aa Sb bx*^{34e} *e Ser.* Horka is a dominant female-sterile mutation on the third chromosome, localized to cytological region 87E (30). When this chromosome is contributed by males, the female progeny show frequent X-chromosome loss leading to production of gynandromorphs (30).

(*iii*) *D. melanogaster y w f*: a standard stock used for scoring maternal X-chromosome loss, as XO male parts will show combinations of the X-chromosome-linked recessive markers (yellow cuticle, white eyes, and forked bristles). This limits us largely to scoring the dorsal half of the body, although the gender of genitals on the ventral abdomen can be determined by their morphology.

Generation of Gynandromorphs. Horka/*TM3 Sb, Ser* males were crossed to *y w f* females, and gynandromorph progeny (≈5% of the total) were identified by their possession of the *y*, *w*, or *f* markers. We made efforts to collect a variety of gynandromorphs with diverse amounts and locations of male tissue.

Scoring Gynandromorphs. To determine the sex of various parts of the fly's cuticle, the external body of each mosaic was divided into 28 sections and each section was separately scored for the proportion of male tissue. These sections included the left eye, left half of the head (excluding eye), right eye, right half of the head (excluding eye), six legs (scored individually), left wing, right wing, left half of the thorax, and right half of the thorax. The abdomen was divided into 14 parts, tergites 1 + 2 (combined, with right and left half of the combined segments scored separately), tergites 3–7 (left and right halves of each tergite scored separately), and left and right halves of genitalia. Except for the genitalia and legs, only the dorsal portion of the body was scored.

The proportion of male tissue in each of these 28 segments was estimated visually to the nearest 25% or 33%, and segments having only a small patch of wild-type or yellow tissue were scored as 0.10. Thus, the percentage of male tissue in each segment was constrained to the values 0, 0.10, 0.25, 0.33, 0.50, 0.67, 0.75, 0.90, and 1.00.

The net proportion of male tissue in each of six major structures (head, legs, wings, thorax, abdomen, and genitals) was then determined by calculating a weighted average of the constituent elements based on a visual estimation of their contribution to the area of the total structure. These data were used in multiple-regression analyses to determine which body parts were significantly associated with the hydrocarbon dimorphism (see *Results*).

Gas Chromatography. Chromatography of individual flies was performed as described (12, 14). Hydrocarbon peaks were identified by comigration with known standards, and absolute quantities of the three hydrocarbons of interest (7-T, 7,11-HD, and 7,11-ND) were estimated by comparing their peak areas with that of an internal *n*-hexacosane standard added to each sample [peak area is proportional to the gram quantity of hydrocarbons (14)].

Courtship Tests. A separate group of 202 *D. melanogaster* gynandromorphs were tested for their attractiveness to *D. simulans* males. As in our previous study (14), gynandromorphs were freshly killed so that their behavior could have no influence on their attractiveness, leaving males with only chemical and visual cues. Flies were aged for 4 days at 24°C on cornmeal food. Each female was then flash frozen in liquid nitrogen [a procedure that removes <5% of the cuticular hydrocarbons (14)] and immediately placed in a food-containing vial. Two 4-day-old virgin *D. simulans* males were introduced into each vial and observed for 20 min. Male courtship behaviors were scored without the observer knowing the precise nature of the mosaic.

As in our previous work (14), we scored three aspects of courtship: courtship latency (time until any male first oriented toward the female and vibrated his wings), number of courtship bouts, and number of attempted copulations (male curls abdomen and attempts to mount the dead female). We also scored the courtship of *D. simulans* males presented with 26 nonmosaic F_1 hybrids of each sex.

Statistical Analysis. In multiple-regression analyses, which involved six independent variables, we corrected significance levels by the standard Bonferroni procedure, dividing the standard significance level ($P = 0.05$) by 6 to produce a threshold significance level of 0.008. Two-tailed tests were used in all cases.

RESULTS

Because mosaics are females who develop male tissue through loss of an X chromosome, we expressed their pheromone constitution by two indices of "maleness," expressed as the difference between the nanogram quantities of the predominant male compound and the two predominant female compounds [these indices are (7-T − 7,11-HD) and (7-T − 7,11-ND) respectively]. For parametric statistical analysis, indices involving differences are preferable to those involving ratios.

Table 1 (bottom) gives the quantities of the three principal hydrocarbons and the maleness indices for the three genotypes of nonmosaic males and females (Horka, *y w f*, and their F_1 hybrid). These hydrocarbon profiles are typical of non-African *D. melanogaster*: females have high levels of 7,11-HD and 7,11-ND, and males lack these compounds but have instead high levels of 7-T.

To roughly summarize the relationship between body-part mosaicism and hydrocarbon profile, we used the procedure of Hall (16) and Jallon and Hotta (18), grouping flies by whether each of the three major tagmata (head, thorax, and abdomen) were completely male, completely female, or a mixture of male and female tissue (Table 1). The "mixed" class for any tagma comprised flies having male tissue in any amount <100%, so that a variety of mosaics may be subsumed within this class. Genital tissue was included as part of the abdomen, so that a fly with a purely female abdomen but with either mosaic or male genitalia would be scored as mixed. We collected individuals from 20 of the 25 possible classes of mosaics.

Table 1 shows that the gender of the abdomen is clearly the major determinant of hydrocarbon profile. (We present below a more accurate multiple-regression analysis, which yields the same conclusion.) Flies with completely male abdomens consistently have high positive values of pheromonal maleness, those with female abdomens have negative values, and those with abdomens of mixed gender have intermediate values. Male and female hydrocarbons are obviously negatively correlated among classes, so that flies having high levels of 7-T also have low levels of 7,11-HD and 7,11-ND.

In contrast, the head and thorax show no obvious relationship between the gender of the cuticle and the relative amounts of male and female hydrocarbons. In most cases, mosaics with abdomens of a single gender have pheromonal indices close to those of nonmosaic F_1 hybrids of the same gender, regardless of the degree of mosaicism in other parts of the body. Thirty-nine mosaics had abdomens (and genitalia) that were

Table 1. Hydrocarbons in different classes of mosaics, pure species, and nonmosaic F_1 hybrids

H	T	A	n	Mean hydrocarbons per fly, ng				
				7-T	7,11-HD	7,11-ND	7-T − 7,11-HD	7-T − 7,11-ND
Mosaics								
F	F	M/F	23	269.50 (53.53)	177.25 (22.26)	143.82 (20.16)	92.25 (52.61)	125.68 (55.28)
F	M/F	F	3	46.03 (8.93)	270.45 (27.10)	188.50 (42.41)	−224.41 (19.15)	−142.46 (42.28)
F	M/F	M/F	17	267.61 (52.60)	156.55 (17.74)	122.46 (12.34)	111.06 (57.15)	145.15 (58.07)
F	M/F	M	3	905.16 (86.21)	14.79 (7.53)	9.44 (5.47)	890.37 (80.11)	895.71 (80.94)
F	M	M/F	4	450.07 (205.75)	126.44 (75.30)	93.32 (48.91)	323.63 (183.89)	356.74 (198.53)
M/F	F	F	17	48.30 (9.06)	251.61 (30.70)	110.57 (26.82)	−203.30 (26.05)	−186.21 (22.29)
M/F	F	M/F	15	207.27 (57.96)	156.84 (24.96)	149.89 (24.67)	50.43 (52.78)	57.38 (53.68)
M/F	F	M	2	360.14 (171.06)	37.84 (0.001)	12.89 (0.68)	322.30 (171.06)	347.25 (171.74)
M/F	M/F	F	13	43.95 (10.36)	231.37 (24.28)	189.79 (17.01)	−187.42 (23.68)	−145.84 (18.18)
M/F	M/F	M/F	56	229.61 (25.97)	140.06 (11.09)	111.97 (8.97)	89.55 (30.60)	117.64 (29.93)
M/F	M	F	1	30.12 —	194.19 —	259.15 —	−164.07 —	−229.03 —
M/F	M	M/F	13	310.97 (65.57)	93.18 (15.98)	71.48 (13.47)	217.78 (66.04)	239.48 (68.75)
M/F	M	M	7	510.85 (37.61)	1.41 (1.41)	0 (0)	509.43 (36.56)	510.85 (37.61)
M	F	F	1	118.46 —	45.86 —	59.57 —	72.61 —	58.89 —
M	F	M/F	2	629.97 (619.05)	327.44 (231.62)	256.39 (151.90)	302.53 (387.43)	373.58 (467.14)
M	M/F	F	2	42.48 (9.20)	202.66 (45.76)	245.76 (69.69)	−160.18 (54.97)	−203.28 (78.89)
M	M/F	M/F	7	241.92 (76.47)	122.04 (14.96)	90.92 (12.56)	119.87 (78.86)	151.00 (82.69)
M	M/F	M	2	438.93 (406.62)	49.41 (49.41)	70.70 (70.70)	389.52 (456.03)	368.24 (477.32)
M	M	F	2	29.14 (10.99)	102.37 (33.39)	103.00 (9.48)	−73.22 (44.38)	−73.85 (20.47)
M	M	M/F	8	355.61 (57.61)	73.29 (30.03)	52.98 (18.74)	282.32 (37.45)	302.63 (45.55)
Nonmosaics								
Females								
y w f			15	42.34 (2.29)	178.12 (10.87)	118.07 (5.06)	−135.78 (9.90)	−75.72 (4.68)
Horka			15	42.58 (5.56)	252.08 (22.14)	136.06 (19.88)	−209.50 (22.01)	−93.48 (17.05)
F_1 (*y w f* ♀ × Horka ♂)			15	38.68 (1.59)	220.90 (20.02)	172.77 (9.74)	−182.27 (9.42)	−134.09 (8.94)
Males								
y w f			15	91.89 (18.18)	0	0	91.89 (18.18)	91.89 (18.18)
Horka			16	456.44 (38.78)	0	0	456.44 (38.78)	456.44 (38.78)
F_1 (*y w f* ♀ × Horka ♂)			15	641.77 (50.43)	0	0	671.77 (50.43)	671.77 (50.43)

As described in the text, each mosaic was classified as to whether each of its three major body segments [head (H), thorax (T), and abdomen (A)] were male (M), female (F), or mosaic (M/F). The two columns on the right give the two pheromonal male indices (see text). Standard errors are in parentheses.

completely female, and these flies had maleness indices nearly identical to those of the nonmosaic F_1 females [for the mosaics (7-T − 7,11-HD) = −183.7, SE = 15.8; (7-T − 7,11-ND) = −161.03, SE = 13.9; compare these to the indices of females at the bottom of Table 1]. Likewise, for the 14 mosaics whose dorsal abdomen, and genitalia were completely male, the maleness indices are close to those of the nonmosaic males [for the mosaics (7-T − 7,11-HD) = 547.2, SE = 77.14; (7-T − 7,11-ND) = 549.6, SE = 78.8; compare to indices of males at the bottom of Table 1]. The *t* statistics show that none of these indices differs significantly from those from the nonmosaic F_1 offspring of the same sex. It is clear from this analysis that the gender of the abdomen is a nearly complete determinant of hydrocarbon profile, and other parts of the fly have little or no effect.

Our scoring of mosaics in this crude fashion does miss some pheromone-producing tissue but not very much. This can be seen by comparing the amount of female hydrocarbons in mosaics having completely male abdomens, as nonmosaic F_1 males produce no 7,11-HD or 7,11-ND. Of 14 such mosaics, 7 had no female hydrocarbons, and the other 7 had low amounts (7,11-HD, mean = 32.7 ng, SE = 12.2; 7,11-ND, mean = 27.9 ng, SE = 19.1). These small amounts could be due to hydrocarbon production elsewhere on the body or to the presence of female abdominal tissue not detected because it was either internal or on the ventral cuticle of the fly.

The proportions of male cuticle among the different body parts are not independent because mosaics usually have a single X-chromosome loss early in development. To distinguish the independent effects of each body part on hydrocar-

Table 2. Multiple regressions of the two pheromonal male indices against the proportion of each body part in the mosaics showing male tissue

	(7-T − 7,11-HD)			(7-T − 7,11-ND)		
	Coefficient	*t* value	*P* value	Coefficient	*t* value	*P* value
Intercept	−187.728	−7.696	<0.0001	−157.273	−6.260	<0.0001
Head	−0.219	−0.567	0.57	−0.382	−0.962	0.34
Thorax	−0.453	−0.722	0.47	−0.537	−0.831	0.41
Wing	1.577	1.981	0.05	1.736	2.117	0.04
Leg	−0.812	−1.337	0.18	−0.823	−1.315	0.19
Abdomen	6.049	14.233	<0.0001*	5.826	13.308	<0.0001*
Genital	0.904	3.027	0.003*	1.078	3.504	0.0006*

*Effects significant using the Bonferroni correction.

Table 3. Courtship of killed mosaic females and nonmosaic F_1 progeny by *D. simulans* males

Head	Thorax	Abdomen	No. of mosaics	Courtship latency, min		Courtship episodes		Copulation attempts	
				Total	Mean/mosaic (SE)	Total	Mean/mosaic (SE)	Total	Mean/mosaic (SE)
Mosaics									
F	F	M/F	28	7	5.36 (1.38)	24	0.86 (0.36)	13	0.46 (0.30)
F	M/F	F	3	0	—	0	0	0	0
F	M/F	M/F	24	13	4.06 (0.73)	30	1.20 (0.46)	12	0.48 (0.33)
F	M	M/F	3	2	4.98 (2.60)	2	0.67 (0.67)	0	0
M/F	F	F	9	0	—	0	0	0	0
M/F	F	M/F	22	8	4.41 (0.85)	19	0.86 (0.36)	0	0
M/F	M/F	F	7	1	11.55 (—)	1	0.14 (0.143)	0	0
M/F	M/F	M/F	53	20	4.58 (1.03)	46	0.87 (0.20)	6	0.11 (0.04)
M/F	M/F	M	2	1	4.90 (—)	5	2.50 (2.50)	4	2.00 (2.00)
M/F	M	F	2	0	—	0	—	0	—
M/F	M	M/F	7	3	6.09 (3.26)	4	0.57 (0.30)	0	—
M/F	M	M	6	3	3.19 (1.28)	10	1.67 (0.92)	9	1.50 (1.02)
M	F	F	1	0	—	0	—	0	—
M	M/F	F	1	0	—	0	—	0	—
M	M/F	M/F	12	5	5.62 (2.40)	15	1.25 (0.62)	2	0.17 (0.17)
M	M	M/F	22	6	5.44 (1.05)	11	0.50 (0.20)	6	0.27 (0.20)
Nonmosaics									
F_1 ♀ (*y w f* ♀ × Horka ♂)			26	1	3.57 (—)	1	0.04 (0.04)	0	0
F_1 ♂ (*y w f* ♀ × Horka ♂)			26	15	5.02 (0.72)	29	1.12 (0.38)	19	0.72 (0.30)

Mosaics were classified as described in Table 1. Three measures of courtship were scored as described in the text.

bon profile (with other parts held constant), we multiply regressed the two "maleness indices" against the proportion of each major body part that was male (the dorsal abdomen and genitalia were considered as separate parts). This analysis (Table 2) shows that only the abdominal and genitalic mosaicism significantly affected the hydrocarbon indices and that, as expected from the analysis in Table 1, these effects are very large. The similarity of results between the two indices involving 7,11-HD and 7,11-ND is not surprising as these quantities are highly correlated among mosaics ($r = 0.92$, $n = 198$, $P < 0.0001$). Their structural similarity also implies that they are made in the same biosynthetic pathway.

We do not have enough mosaics to determine which part of the abdomen is most closely associated with hydrocarbon production. However, both anterior (nos. 1–4) and posterior (nos. 5–7) tergites contribute independently and additively to the hydrocarbon dimorphism (data not shown).

Table 3 summarizes the courtship behavior of live *D. simulans* males presented with various mosaics, the latter again sorted by gender of the three major tagmata. For comparison, we also show at the bottom of the table courtship behaviors of *D. simulans* males presented with nonmosaic male and female offspring from the same cross.

With two exceptions, only mosaics having male abdominal tissue receive courtship from *D. simulans* males (Table 3). Among the nonmosaic F_1 progeny, males were subject to frequent courtship and attempted copulation, while females were virtually ignored. Likewise, courtship of the mosaics was limited almost completely to those individuals with male abdominal cuticle, who, as we have seen, are the only mosaics producing appreciable amounts of 7-T. Among the 23 mosaics with a completely female abdomen, only 1 (a proportion of 0.043) experienced a single courtship episode, and there were no attempted copulations. Of 171 mosaics with an abdomen of mixed gender, however, 64 were courted at least once (0.37) and 15 (0.09) were subject to attempted copulation. Finally, of 8 mosaics with a completely male abdomen, 4 (0.50) were courted, and 3 (0.38) were subject to attempted copulation. The average number of courtship bouts per vial shows the same increase with increasing abdominal maleness, with values of 0.04, 0.88, and 1.89, respectively, for the three classes described above, as does the number of copulation attempts per vial (0, 0.23, and 1.62, respectively). The role of the head and thorax in inciting courtship is negligible, as can be seen by comparing the behavior of males toward mosaics having abdomens of the same gender but heads or thoraxes of different gender (e.g., class F, F, M/F vs. class M, M, M/F in Table 3).

The independent effect of mosaicism of each body part on sexual attractiveness to males was estimated by multiple regression (Table 4). Courtship latency is not affected by mosaicism of any segment. (Since only one fly with a female abdomen was courted, this comparison involves flies with mosaic versus male abdomens.) For the other two courtship characters, however, only the abdomen and genitalia have highly significant effects, with more male tissue eliciting more courtship.

D. simulans males can therefore be induced to court *D. melanogaster* mosaics if these mosaics have male tissue in the

Table 4. Multiple regression of three measures of courtship intensity (*D. simulans* males) against the proportion of each body part in the target mosaics showing male tissue

	Courtship latency			Courtship bouts			Copulation attempts		
	Coefficient	*t* value	*P* value	Coefficient	*t* value	*P* value	Coefficient	*t* value	*P* value
Intercept	5.090	4.261	<0.0001	0.240	1.065	0.29	−0.111	−0.758	0.45
Head	0.010	0.595	0.55	−0.003	−0.878	0.38	−0.003	−1.106	0.27
Thorax	0.028	0.736	0.46	−0.009	−1.438	0.15	−0.002	−0.463	0.64
Wing	−0.026	−0.598	0.55	−0.003	−0.481	0.63	−0.001	−0.292	0.77
Leg	0.012	0.426	0.67	0.008	1.557	0.12	0.001	0.366	0.71
Abdomen	−0.029	−1.487	0.14	0.015	3.386	0.0009*	0.009	3.451	0.0007*
Genital	0.0013	0.103	0.92	0.008	3.295	0.0012*	0.005	2.877	0.0045*

*Effects significant using the Bonferroni correction.

body parts associated with pheromone dimorphism. This in turn buttresses our previous findings, in other species of the group, that cuticular hydrocarbons contribute to sexual isolation.

DISCUSSION

As only the abdomen and genitalia have significant effects on hydrocarbon profile, our results clearly suggest that the abdomen is the key site regulating production of sex-specific pheromones in *D. melanogaster*. (The effect of the genitals may indicate not that they produce hydrocarbons but that they mark the gender of ventral abdominal tissue, an area that cannot be studied with our mutant markers.) Of course, the large effect of the abdomen does not prove that this segment actually synthesizes or secretes the hydrocarbons, for it may harbor only a sex-specific signal that regulates synthesis elsewhere in the body. There is some evidence that the abdominal integument is indeed the site of pheromone production in the housefly (10, 11, 22); the abdomen is also the major site of pheromone emission (although not necessarily synthesis) in the mosquito *Culicoides nubeculosus* (21). One should not assume, however, that all dipterans share the same developmental origin of these compounds. In houseflies, for example, the ovary is required for production of female hydrocarbons (31) but this is not true in *D. melanogaster* (32).

Jallon and Hotta (18) speculated that cuticular hydrocarbons may be synthesized by the fat bodies, a possible site of fatty acid metabolism. In the housefly, however, elongation of fatty acids, an essential step in the synthesis of the female pheromone (*Z*)-9-tricosene, occurs at far higher rates in abdominal integument than in the fat body (10). Settling this issue in *Drosophila* will require internal tissue markers such as those used in studies of brain mosaicism (33).

The identification of the abdomen as the key site of pheromone dimorphism explains the results of three previous studies (16–18) showing that males court primarily those mosaics with female abdomens and supports the idea, suggested in all of these studies, that the attraction to the abdomen is due to cuticular hydrocarbons.

Wicker and Jallon (34) observed that females decapitated less than a day after eclosion subsequently produce very little 7,11-HD or 7,11-ND, that decapitated males produce somewhat reduced amounts of 7-T, and that decapitated individuals of both sexes retain the juvenile hydrocarbons that usually disappear soon after eclosion. These authors concluded that the head must be involved in pheromone production. This does not necessarily contradict our observation that the gender of the head plays no role in the sexual dimorphism of hydrocarbons. Head tissue (most likely in the brain) may produce a nonspecific hormone affecting target tissue in the abdomen, and it may be the gender of the target tissue that determines which pheromones are produced (32, 34).

The attraction of *D. simulans* males to gynandromorphs with male abdomens not only supports previous observations that 7-T induces courtship of *D. simulans* males but also suggests that the difference between *D. simulans* and *D. melanogaster* female pheromones contributes to sexual isolation (14). It is important to note, however, that the species difference in cuticular pheromones cannot completely account for all sexual isolation. *D. simulans* males, for example, do not court dead *D. melanogaster* males nearly as intensely as they court dead females of their own species, despite the fact that both courted genotypes have similar amounts of 7-T and other large hydrocarbons (5, 14). This behavioral difference may be due to either the abdominal morphology of the dead males or pheromones not detected by our assay, such as the male-specific compound *cis*-vaccenyl acetate known to inhibit male courtship in *D. melanogaster* (35, 36). Nevertheless, the mosaic studies show that female morphology cannot attract *D. simulans* males in the presence of a pheromonal difference, while a pheromonal similarity can partially overcome the effect of the morphological difference between the sexes. As is undoubtedly the case for most species, sexual isolation in *Drosophila* involves interactions between morphological, behavioral, and chemical differences among species.

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